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Anti-Bacterial Effect Of Hvpc In Vivo

Marc Campolo
Seton Hall University

ANTI-BACTERIAL EFFECT OF HVPC *IN VIVO*

by

Marc Campolo, MA, PT, SCS

Dissertation Committee:

Dr. Genevieve Pinto-Zipp, Chair

Dr. MaryAnn Clark

Dr. Alma Merians

Dr. Chandra Williams

Approved by the Dissertation Committee:

Genevieve Pinto-Zipp Date 12-15-99

[signature] Date 12-15-99

Alma Merians Date 12/6/99

Chandra Williams Date 12-15-99

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DEDICATION

I dedicate this work to my wife, Debbie, and my sons, Max, Luke, and Jake, whose unselfish sacrifice and understanding made this possible. It is your love, and my love for you that makes this work and life in general worthwhile.

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Abstract

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PURPOSE: A historical review of the literature reveals that low intensity direct current (LIDC) is effective in the treatment of infected wounds. Since the 1970'2, high voltage pulsed current (HVPC) stimulators have been used for the same purpose based on the assumption that they have the same physiological effects as LIDC. To date; however, there is insufficient research to support clinical use of HVPC for infected wounds. The purposed of this pilot study was to determine whether HVPC has an inhibitory effect on bacteria *in vivo* in order to provide evidence to support the clinical use of HVPC stimulation in the treatment of infected wounds.
SUBJECTS: An animal model was used in order to avoid any of the confounding effects associated with the use of human subjects. All the rules and regulations set forth by the Institutional Animal Care and Use Committee were strictly adhered to. IRB approval was also obtained. The subjects consisted of 12 New Zealand rabbits of equivalent size, weight and age.
METHODS AND MATERIALS: The animals were randomly assigned to either an experimental group (EXP=7) or a control group (CON=8). Each animal was anesthetized and a full thickness wound (2cm by 3cm) was made on their backs, which was then infected with 1 m. of 1 X 10^7 Staphylococcus Aureus solution. The wound was then covered with a sterile dressing. Bacterial colony counts, measured in CFU's, were used to establish both the initial and final level of infection. Data were acquired from wound cultures obtained 1 day post infection (initial). Treatment was initiated the following day and continued for six consecutive days. Electrodes were placed on all of the animals; however, only the EXP group received electrical stimulation. The parameters chosen were consistent with those recommended clinically: the waveform was monophasic and twin peak in shape, the phase duration was 75 usec, the pulse rate was 7100 pps, the amplitude was the highest obtainable without causing muscular contraction (not to exceed 100V), and the current modulations was continuous.
RESULTS: A Mann-Whitney U test (Table 3) conducted compared the difference between the % change of the bacterial counts, revealed that there was no statistically significant difference between the EXP and CON groups from the initial to final condition (V=18, P=0.246). Although statistical significance was not achieved, further assessment of the data revealed to interesting trends. First, it was noted that the EXP group consistently exhibited a substantial decrease in their bacterial counts across all subjects and almost complete abolition in most subjects (mean decrease -97.4%, SE 1.36). Conversely, consistent results were not observed in the CON group; although some subjects did exhibit a marked decrease in their respective bacteria count, others exhibited a substantial increase resulting in a mean increase of 22.5% (SE 75.56). Second, when converting the data into ranks, the ranked data appears to exhibit an interesting general tendency. The mean rank of the CON group increased from 6.69 to 8.75, while that of the EXP group decreased from 9.50 to 7.14.
CONCLUSION: Although statistical significance may not have been achieved, based upon the noted trends in the data, the author suggests that HVPC may have contributed to the demonstrated positive trends of exhibiting bactericidal effects in acute wounds of animals. Further studies using human subjects are necessary to truly establish the efficacy of the anti-bacterial effects of HVPC.

CHAPTER I

INTRODUCTION

Pressure ulcers are a common but serious problem that affects acute care, nursing home, and home care populations. It has been reported that this condition affects 9 percent of all hospitalized patients and 23 percent of all nursing home patients and the total national cost has been estimated to be $1.335 billion dollars (Bergstrom, et al, 1994). Additionally, several populations may be at higher risk, including quadriplegic patients (60 percent prevalence), elderly patients who sustained femoral fractures (60 percent prevalence), and critical care patients (41 percent prevalence). Prompt and effective treatment can minimize the deleterious effects of pressure ulcers such as pain and disfigurement; however, if the condition becomes chronic, it can be difficult to treat and result in prolonged hospitalization.

A chronic wound is defined as one that deviates from the expected sequence of repair in terms of time, appearance, and response to aggressive and appropriate treatment (Sussman & Bates-Jensen, 1998). Delayed healing can result from any combination of intrinsic, extrinsic or iatrogenic factors; intrinsic factors affecting chronic wound healing include aging, chronic miscare, circulatory disease, malnutrition and neuropathy; extrinsic factors include medication and immune suppression, irradiation, psychophysiologic stress, and infection; and iatrogenic factors include ischemia and inappropriate wound care management resulting in trauma to the wound.

Reports of wound management by physical therapists have appeared in the physical therapy literature for more than three decades (Sussman & Bates-Jensen, 1998). As a highly respected member of the wound-care team, the physical therapist assists in all

aspects of wound care: including, debridement, dressing selection and application, and recommending strategies to relieve or redistribute pressure for those confined to bed or wheelchair or for the ambulatory individual with an insensate foot. Additionally, physical therapists also provide a unique function, they are skilled in the use of physical agents (heat, light, sound and water), therapeutic exercise, and electrotherapeutic modalities, all of which have benefits to offer the patient in contributing to wound healing strategies (McCulloch, 1998). However, recently, the use of electrotherapeutic modalities has become somewhat controversial following the Health Care Finance Administrators (HCFA) July 14, 1997 decision to deny payment for the use of electrical stimulation in the treatment of wounds. HCFA's non-coverage decision was based on two new requirements for Medicare coverage of electrical stimulation. First, that a treatment such as electrical stimulation under consideration must be "markedly superior" to other treatments for similar conditions, and second, that devices such as electrical stimulators used in a particular treatment receive FDA approval for the specific use under consideration, i.e. wound treatment (SCE Newsletter, 1997).

Background of the Problem

Electrical stimulation as a means of enhancing healing was practiced as far back as the seventeenth century (Davis and Ovington, 1993); however, not until the mid 1900's was research produced supporting the idea that therapeutic doses of electrical current can augment healing of chronic wounds due to the bactericidal effects of electrical current and the stimulation of granulation tissue growth (Alvarez, Mertz, Smerbeck & Eaglstein, 1983; Byl, et al, 1994; Calrley & Wainapel, 1985; Castillo, et al,

1995; Gault & Gatens, 1976; Leffmann, et al, 1994; Lundenberg, et al, 1992; Mulder, 1991; Pomeranz, et al, 1993; Wolcott, Wheeler, Hardwicke & Rowley, 1969).

Two types of electrical stimulators have been traditionally used for accelerating wound healing: low voltage direct current (LVDC) stimulators and high voltage pulsed current (HVPC) stimulators (Nelson & Currier, 1991). LVDC stimulators produce a continuous uninterrupted unidirectional flow of charged particles, commonly referred to as "galvanic" current, which typically produces less than 100 volts of peak voltage. HVPC stimulators produce a unidirectional monophasic pulsed current with peak amplitudes of 100 to 500 volts, with a wave form that is typically twin-peak in shape and designed to last for a short period of time (5 to 100 microseconds). HVPC stimulators were initially classified as "high voltage galvanic stimulators" by their manufacturers due to the nature of their monophasic pulse. This misnomer has led to some confusion and inappropriate use of HVPC stimulators because clinicians assumed that these units had similar characteristics and effects, and therefore, the same uses as the "low voltage galvanic stimulators". Many clinical applications of HVPC have been suggested, including its use to enhance wound healing, but the basis for this is derived from the results of research performed using LVDC (Nelson & Currier, 1991; Sussman & Bates-Jenson, 1998).

Bacterial burden is one of the extrinsic factors related to delayed wound healing. The use of electrical stimulation (both HVPC and LVDC) to inhibit or destroy wound pathogens *in vitro* and *in vivo* has been documented extensively (Barranco, Spadaro, Berger, & Becker, 1974; Guffey & Assmussen, 1989; Kincaid & Lavoie, 1989; Ong, Laatsch, & Kloth, 1994; Rowley, 1972; Rowley, McKenna, & Chase, 1974; Szuminsky,

Albers, Unger, & Eddy, 1994; Wheeler, Wolcott, & Morris, 1971). Rowley (1972) reported bactericidal effects *in vitro* on Escherichia coli B growth rates using cathodal microamp direct current. Similarly, Rowley, McKenna, & Chase (1974) demonstrated a bactericidal effect on Pseudomonus aeruginosa on rabbit skin wounds using LVDC. A study by Barranco, Spadaro, Berger, & Becker (1974) revealed that cathodal LVDC decreased growth rates of staphylococcus aureus in infected rat and rabbit femurs.

With regards to efficacy of HVPC bactericidal effects, there have only been two studies to date that demonstrated positive effects, and both have been conducted *in vitro*. Kincaid & Lavoie (1989) reported that the growth of three microorganisms commonly found in human wounds was inhibited *in vitro* at both the anode and cathode when exposed to 250 volts of HVPC for two hours. More recently, Szuminsky, Albers & Eddy (1994) reported similar results on four different species of bacteria when subjected to 500 volts of HVPC for 30 minutes. The consensus with these studies are that the amplitudes used would be intolerable if used on infected wounds in humans and *in vitro* studies do not take into account the circulatory effects of HVPC.

The proposed mechanisms by which electrical stimulation exhibits its anti-bacterial effects include electrolysis, galvanotaxis, and alteration of tissue pH (Ong, Laatsch, & Kloth, 1994). Electrolysis is the death of the bacteria resulting from the direct action of the electric current. Wheeler, Wolcott, & Morris (1971) postulated that the bactericidal effect of continuous cathodal current might be due to either the depletion of bacterial substrates or disruption of intracellular metabolic processes resulting in the death of the organism by a direct electrolytic effect.

Galvanotaxis is the attraction of the cells of repair to the anode or cathode (Cooper & Schliwa, 1985). Neutrophils, lymphocytes, platelets, and macrophages are early responders to injury and start the inflammatory response. Research has demonstrated that polarity influences the motility of various cells. Neutrophils have been observed to be attracted to the negative pole if the wound is infected and to the positive pole if not infected (Fukoshima & Sends, 1953). Lymphocytes and platelets are attracted to the negative pole (Bourguignon & Bourguignon, 1989), whereas macrophages and fibroblasts are attracted to the positive pole (Orida & Feldman, 1982). It has been suggested that perhaps the documented anti-bacterial effects of electrical stimulation were the result of galvanotaxic attraction of phagocytic macrophages and leukocytes to infected tissues rather than from detrimental effects of pathogens caused by electrolysis or altering the tissue pH (Ong, Laatsh, & Kloth, 1994; Rowley, McKenna, & Chase, 1974).

Electrochemical effects occur as a result of the polarizing effects of continuous use of direct current. Unidirectional current flow causes the migration of ions from dissociated salts. Within the tissue, positively charged ions, such as sodium (Na+), migrate toward the cathode; whereas, negatively charged ions, such as chloride (Cl-), migrate toward the anode. The result is a chemical reaction; the formation of hydrochloric acid under the anode and the formation of sodium hydroxide under the cathode. This resulting chemical reaction changes the pH of the tissue, which can lead to cellular death. However, this condition only occurs with LVDC. Due to the nature of its waveform, HVPC has a very low average current, and as a result there is very little, if any, chemical reaction under the electrodes (Newton & Karselis, 1983). Based on current

literature, it is doubtful that the bactericidal effects of HVPC is a result of alteration of the tissue pH.

Problem Statement

Clinically, wound healing is impeded where infection is present. The purpose of this study was to determine if HVPC, used at amplitudes human patients can tolerate clinically, has an effect, *in vivo*, in reducing the viability of an infecting microorganism in wounds; thereby, positively affecting one of the extrinsic factors contributing to delayed wound healing.

Definitions

Amperage the unit of current being the ampere (A), is defined as the rate at which electrons move past a certain point. A milliampere (mA) is one thousandth of an ampere.

Amplitude refers to either the voltage or the current intensity of an electric current. Voltage is a measure of the force of the flow of electrons and amperage is the measure of the rate of flow of the current. When voltage is turned up, the current will also go up, and vice versa. Some stimulators provide a readout of voltage and some a readout of current.

Anode the positive electrode.

Antibacterial any agent, physical or chemical, that eliminates living organisms pathogenic to host.

Bactericidal destructive to or destroying bacteria

Biphasic wave forms bi-directional current flow. Biphasic waves are such that the polarity is constantly changing. They are opposite at any moment in time. However, the wave form can be biased so that one polarity is emphasized. Symmetric biphasic wave forms are balanced and have no net polarity. Asymmetrical biphasic wave forms are unbalanced and exhibit a polarity based on the bias.

Capacitive Coupling capacitively coupled electrical stimulation involves the transfer of electric current through an applied surface electrode pad that is in wet (electrolytic) contact (capacitively coupled) with the external skin surface and/or wound bed.

Cathode the negative electrode.

Chronic Wound one that deviates from the expected sequence of repair in terms of time, appearance and response to aggressive and appropriate treatment.

Decubitus Ulcer an ulcer, initially of the skin, due to prolonged pressure, usually in a person who is lying down. However, pressure ulcers or sores may occur at any site (e.g., on the buttocks of patients confined to wheelchairs). The most common sites are over bony promineneces (i.e., the sacrum, heels, trochanter, lateral malleoli, and ischial areas). The combination of pressure, shearing forces, friction, and moisture lead to the death of tissue due to the lack of blood supply, if not treated vigorously, the ulcer will progress from a simple erosion to complete involvement of the deep layers of the skin and may eventually extend to the underlying muscle and bone tissue.

Electrical Stimulation for Wound Healing is defined as the use of a capacitive coupled electrical current to transfer energy to a wound. The type of electricity that is transferred to the target tissue is controlled by the electrical source.

Galvanotaxis unidirectional electrical current flow in the tissues attracts the cells of repair. An example would be neutrophils, which are granulocytic leukocytes that function as phagocytic cells that proliferate in the hypoxic acidotic environment and produce superoxide to fight bacteria. Neutrophils are attracted to the cathode if the wound is infected (Fukoshima & Sends, 1953).

High Voltage Pulsed Current (HVPC) is associated with a class of electrotherapeutic devices that have a "twin-peak" monophasic wave form with a fixed duration in the microsecond range (up to 200 usec) and a voltage greater than 100 volts. There is a long interpulse interval between pulses that results in a low average current.

In vitro in glass, as in a test tube. An *in vitro* test is one done in the laboratory, usually involving isolated tissue, organ, or cell preparations.

In vivo in the living body or organism. An *in vivo* test is one performed on a living organism.

Low Volt Direct Current (LVDC) is continuous, uninterrupted, unidirectional current. Direction of the flow is determined by the polarity selected. This form of current has been traditionally called galvanic current. In general, low voltage generators produce voltages in the range of 60 to 100 volts.

Microamp Direct Current is a direct current with an amplitude of less than one millivolt.

Monophasic wave forms have uni-directional current flow. Monophasic waves are such that at one electrode the polarity is positive and the other is negative.

Polarity refers to the property of having two poles that are oppositely charged. The positive pole is called the anode and the negative pole is the cathode. The positive pole lacks electrons and attracts them from the negative pole. Polarity can be chosen or emphasized for biological effects.

Power analysis the power of a hypothesis test equals the probability of detecting a particular effect, that is, of rejecting the null hypothesis. Power analysis is used to determine the appropriate sample size for a projected experiment.

Pressure Ulcer any lesion caused by unrelieved pressure resulting in damage of underlying tissue. Pressure ulcers usually occur over bony prominences and are graded or staged to classify the degree of tissue damage observed.

Wave Form different types of current have different characteristic wave forms. Wave forms are the graphic representations of a current on a current/time or voltage/time plot. Waveforms are classified by the direction of current flow. Current flow is either unidirectional or bi-directional.

Hypothesis

HVPC will exhibit an antibacterial effect *in vivo* at levels that humans can tolerate. The mechanism of action is postulated to galvanotaxis and not electrolysis.

Rationale

The studies conducted by Kincaid & Lavoie (1989) and Szuminsky, Alber, Unger, & Eddy (1994) *in vitro* suggests that electrolysis occurs at voltages greater than 250

volts. Conversely, Guffey & Asmussen's (1989) *in vitro* study revealed that HVPC amplitudes less than 160 volts had no such effect. This project used voltages less than 100 volts, so it is unlikely that if HVPC does exhibit an antibacterial effect, the mechanism of action would be electrolysis. Additionally, because HVPC does not affect pH levels (Newton & Karselis, 1983), alteration of pH cannot be considered as the probable cause of the antibacterial effect.

Previous investigations support the efficacy of LVDC in wound healing for both stimulation of granulation tissue effects (Wolcott, Wheeler, Hardwicke, & Rowley, 1969; Gault & Gatens, 1976; Carley & Wainapel, 1985) and its antibacterial effects (Barranco, Spadaro, Berger, & Becker, 1974; Rowley, McKenna, & Chase, 1985). Clinical studies performed by Feeder & Kloth (1985), Kloth & Feeder (1988), Feeder, Kloth, & Gentzkow (1991), and Unger, Eddy, & Raimastry (1991) provide evidence supporting the efficacy of HVPC in the stimulation of granulation tissue. Both LVDC and HVPC stimulators exhibit polar capabilities (the ability to create a positive or negative electrical field) due to their monophasic pulse configuration. It is these polar effects that appear to give LVDC and HVPC their granulation tissue stimulation effects as studies using biphasic waveforms achieved the best wound healing effects when the biphasic wave form is asymmetrical and biased so that the polarity at one pole predominates (Baker, Chambers, DeMuth, & Villar, 1997; Lundenberg, Eriksson, & Malm, 1992; Stefanouska, et al, 1993). It would be reasonable to assume that if a HVPC stimulator is able to provide a sufficient electrical field to stimulate granulation tissue growth due to its polar capabilities, HVPC should be able to provide a strong enough electrical field to stimulate galvanotaxis and the resulting antibacterial effects.

Need for Study

All studies to date that have been performed to determine the antibacterial effects of HVPC have been conducted *in vitro*, and the authors agree that in order to truly establish the efficacy of HVPC in the treatment of infected wounds, studies conducted *in vivo* are necessary (Guffey & Asmussen, 1989; Kincaid & Lavoie, 1989; Szuminsky, Albers, Unger, & Eddy, 1994). The *in vitro* studies on bacterial inhibition deal mainly with the electrolytic aspects of electrical stimulation, while an *in vivo* study would be able to determine what effect HVPC stimulation may have on the galvanotaxic attraction of phagocytic macrophages and leukocytes and their role in inhibiting bacterial growth. Additionally, HCFA's July 1997 decision to deny payment for the use of electrical stimulation in the treatment of wounds had deprived many patients of critical treatment they had been receiving. Although an injunction was awarded to the APTA on September 1997, controversy with regards to this treatment persists as a final rule on this decision has yet to be made. A positive outcome of this project may lend support to the clinical use of electrical stimulation in wound care.

CHAPTER II

REVIEW OF THE LITERATURE

The Effects of LVDC and HVPC on Granulation Tissue

The mechanism by which electrical stimulation promotes wound healing is not yet clearly understood, however, the most widely accepted hypotheses are, 1) the "current of injury", 2) cellular level responses, and the 3) bactericidal effect of electrical stimulation. In 1962, Becker et al, conceptualized the existence of a direct current electrical system controlling tissue healing. They stated that the electrical balance of the body is disturbed in an injury resulting in a shift in the current flow and a change in the DC potential of the tissue, referred to as the "current of injury". The authors proposed that the DC electrical potential initially responsible for triggering the healing process was positive, and placement of the anode directly on the wound would facilitate the healing process. Davis & Ovington (1993) stated that investigations of wounded skin demonstrated the existence of natural bioelectrical currents, the exterior layers of the skin being electronegative with respect to the inner layers. If electrical signals play a role in the stimulation of wound repair, then exogenous applications of electrical current to chronic wounds could be expected to mimic the body's bioelectrical currents and enhance the tissue healing process.

Accelerated healing may be related to the cellular responses to electrical stimulation, especially fibroblasts, which have been shown to be fundamental in the process of wound repair as they build the collagen matrix known as granulation tissue. Cruz, Bagron, & Suarez (1989) performed experimental studies on domestic pigs, evaluating the effect of HVPC on the rate of healing of full thickness thermal burns and

found a significantly faster rate of wound contraction and a higher fibroblast response in the stimulated wounds. Bourguignon & Bourguignon (1991) exposed human fibroblast cell cultures to HVPC and concluded that the rates of both protein and DNA synthesis could be significantly increased by exposure to electrical fields; however, at intensities greater that 250 volts an inhibitory effect was noted. Cheng, et al (1982) revealed that direct currents ranging from 10 uA to 1000 uA increase ATP concentration in tissue, stimulating amino acid incorporation into proteins of rat skin, contributing to increases in protein synthesis. Reich, Cazzaniga, Mertz, Kerdel & Eaglstein (1989), who examined the effects of electrical stimulation on mast cells (which are associated with a variety of pathologic skin conditions in humans, including ulcers) in acute wounds on pathogen-free pigs, reported a significant reduction in the number of mast cells seen when the wounds were electrically stimulated. This reaction may be related to a decrease in either proliferation or migration of these cells and may prove to be a valuable therapeutic technique. The above findings indicate that protein, DNA and ATP synthesis, as well as the migratory capacity of epithelial and connective tissue cells involved in repair and regeneration can be affected by an electrical field.

HVPC became popular in the 1970's because it did not have any measurable chemical or thermal reactions under the electrodes, reducing or eliminating all known side effects and precautions associated with LVDC (Alon, 1987). The misnomer of high voltage "galvanic" stimulation was applied to this type of stimulator due to its monophasic wave form, which may have misled clinicians to assume that the mechanisms of action as well as the physiological effects of HVPC were the same as those attributed to LVDC, commonly called "galvanic stimulation". The many clinical

applications of HVPC suggested by manufacturers, including its use to enhance wound healing, were derived from the positive results of research performed using LVDC.

Since 1968, several human clinical studies have demonstrated the effectiveness of LVDC when used to promote the healing of chronic wounds. Assimacopoulos (1968) performed an animal study using rabbits to determine if negative direct electrical current accelerates the epithelization and healing process of a wound. The results revealed that the influence of negative electric current shortened the time of healing by 25%.

In 1969, Wolcott, Wheeler, Hardwicke, and Rowley conducted a study on 67 patients presenting a total of 83 ischemic skin ulcers. LVDC was applied directly to 75 ischemic skin ulcers for an eighteen-month trial. The electrical stimulation produced a mean healing rate of 13.4% per week, and 34 ulcers healed completely. The authors reported that eight patients presented with bilateral ulcers of comparable size and location which could qualify as a control to the treated counterpart. In this subgroup, the treated ulcers healed at a rate of 27% per week compared with 5% per week for the controls. Interestingly, the protocol used negative polarity until asepsis was obtained and then switched to positive polarity. This is consistent with the clinical protocol used presently.

Gault and Gatens (1976) conducted a study on 76 patients who had 106 ischemic skin ulcers. Six patients had bilateral symmetrical ulcers that were closely matched in size, shape, position, and general appearance. The results of this study revealed that 100 ischemic skin ulcers treated with LVDC healed at a rate of 28.4% per week. With regards to the six patients with bilateral ulcers the mean healing ratio of the control ulcers was 14.7% per week compared to 30% per week of the treated counterpart.

Initial studies conducted examining the effects of electric stimulation on granulation tissue had lacked sufficient controls. Recognizing this limitation, Carley and Wainapel in 1985 conducted a study on 30 inpatients. Subjects were paired according to age and diagnosis and wound etiology, location, and approximate size. One member of the pair was randomly assigned to receive the LVDC protocol while the control used conventional wound therapy. The results suggest a 1.5 to 2.5 times faster healing in the experimental group.

More recently, Mulder (1991) conducted a randomized double blind study of electric stimulation with 59 patients representing 67 open wounds of pressure, vascular and surgical etiology. The stimulator used in this project was a low-intensity, pulsed, direct current unit. Different from LVDC in that the current is interrupted versus continuous, similar in that it exhibits polar capabilities. The authors reported that after four weeks of treatment, the electrical stimulation group showed a 56% decrease in size with only a 33% decrease in size in the sham treatment group.

Research to support the hypothesis that HVPC promoted the healing of chronic wounds was not conducted until the mid 1980's. Prior to that time, support for the clinical use of HVPC in the treatment of chronic wounds was extrapolated from LVDC research.

In 1984, Akers and Gabrielson compared three treatment procedures of decubitus ulcers: 1) whirlpool bath , 2) combination of whirlpool bath and HVPC, and 3) HVPC alone. The authors stated that the comparisons of the different techniques indicated that the greatest rate of changed wound size occurred in those patients who received HVPC

alone. The next best rate of change occurred in the combination group, and the least with the whirlpool alone group.

Feeder and Kloth (1985) published a study of eight patients with Stage IV decubitus ulcers. The patients in the treatment group completely healed in a mean of 7.3 weeks, whereas the patients in the control group had an increase in the wound size on the average of 13.8% in a mean of 10.6 weeks.

Alon (1986), in a published abstract, "Diabetic Ulcer Healing Using High Voltage TENS", reported that twelve of fifteen diabetic patients with dermal ulcers had complete healing after a mean of 2.6 months of treatment with HVPC.

A study by Kloth and Feeder (1986) was conducted on 16 patients to determine whether high voltage electrical stimulation accelerates the rate of healing of dermal ulcers. Subjects were randomly assigned to treatment and control groups. The authors reported that the treated ulcers healed completely in an average of 7.5 weeks, whereas, the control group increased in size by 28.9% in an average of 7.4 weeks. Interestingly, when three patients were crossed over from the sham to active treatment group they healed at an average of 38% per week.

More recently, in a published abstract, Unger, Eddy, and Raimastry (1991) reported their work assessing the efficacy of treatment with HVPC on wound healing using a controlled double blind research design. Seventeen patients having pressure ulcers were randomly assigned to either an HVPC or placebo group. The results revealed that of the nine patients receiving HVPC treatment, eight patients were healed. Conversely, of the eight patients receiving sham treatment, only three were healed. The

authors stated that the proportion of patients healed with HVPC was 2.4 times higher than those without such therapy.

Feeder, Kloth and Gentzkow (1991) also conducted a randomized, double blind, multicenter study comparing healing of chronic dermal ulcers treated with HVPC to those treated with sham electrical stimulation. Forty-seven patients were randomly assigned to either the treatment group or the control group. The results of their study indicated that pulsed electrical stimulation had a beneficial effect on healing of Stages II, III, and IV chronic dermal ulcers.

In 1993, Mawson et. al., conducted a study to determine whether HVPC could increase sacral transcutaneous oxygen tension in spinal cord injured persons lying prone and supine. The authors stated that previous research indicates that spinal cord injured patients had lower sacral transcutaneous oxygen tension in these positions, which may be related to the high incidence of pressure ulcers in these patients. Their results indicated that HVPC can reliably increase the sacral oxygen levels of spinal cord injured persons.

Most recently in 1996, Baker, Rubayi, Villar and Demuth conducted a study to evaluate the effect of stimulation waveform and electrode placement on wound healing. Eighty patients with spinal cord injury and one or more pressure ulcers, for a total of 185 ulcers, received 45 minutes of stimulation daily. Comparisons were made between asymmetric biphasic waveform, symmetric biphasic waveform, microcurrent stimulation, or sham. Their results showed significantly better healing rates in the asymmetric biphasic waveform.

Similarly Baker, Chambers, DeMuth, and Villar (1997) conducted a study to evaluate the effects of two stimulation waveforms on healing rates in patients with

diabetes and open ulcers. This study enrolled 80 patients with open ulcers who received stimulation with either an asymmetric biphasic or symmetric biphasic square-wave pulse. The results revealed that stimulation with the asymmetric stimulation enhanced healing by nearly 60% over the control rate of healing, whereas stimulation with the symmetrical wave did not increase the healing rate when compared with the control subjects.

The latter two studies cited are of interest because although the asymmetrical biphasic waveform is different from LVDC and HVPC waveforms in that it has two phases, this waveform does have minimal polar capabilities similar to that of both the LVDC and HVPC waveforms. Thus these findings may lend some support to the notion that the polar capabilities of a waveform play a role in healing capabilities.

Although there have been conflicting results from animal research (Brown & Gogia, 1987; Brown, Gogia, Sinacore & Menton, 1995; Brown, McDonnel & Menton, 1989; Cruz, Banron & Suarez, 1989), additional studies performed on human subjects have supported the efficacy of both LVDC and HVPC in the augmentation of tissue repair.

Bactericidal Effects of Electrical Stimulation

Increased bacterial burden has also been associated with delayed wound closure. Several studies have documented the efficacy of LVDC in inhibiting or destroying wound pathogens *in vitro* and *in vivo* (Alvarez, Mertz, Smerbeck & Eaglstein, 1983; Rowley, 1972; Rowley, McKenna & Chase, 1974). Rowley (1972) noted that stimulation with the negative pole using LVDC caused a decrease in the growth rate of Escherichia coli in an *in vitro* study. Further studies by Rowley, et al (1974) demonstrated a growth retardation of Pseudomonas aeruginosa in rabbit-skin wounds when the cathode of LVDC was

applied to the wound sites. Barranco, et al (1974), using the negative pole of LVDC applied to Staphylococcus aureus infected rabbit femurs, observed a decrease in growth rate of the organisms following one hour of stimulation. Bolton, Foleno, Means, and Petrucelli (1980) studied the effects of LVDC on intact human skin inoculated with Staphylococcus epidermidis. The results revealed that bactericidal activity was exhibited in the 13 subjects included in this study, and that the bactericidal activity for this species was associated with the positive polarity. Another human study by Fakhri and Amin (1987) showed bacterial killing without antibiotics in resistant burn wounds. The researchers studied 20 burn patients, some of whom cultured positive for Pseudomonas, E-coli, and Staphylococcus aureus. LVDC stimulation was applied to the burns for 10 minutes, twice a week until the burn was healed. It was reported that all of the wounds healed. These findings collectively appear to support the use of LVDC in the treatment of infected wounds.

The use of HVPC to promote healing of decubitus ulcer and surgical wounds by decreasing the bacterial burden was initially based on the results of studies using LVDC. Kincaid and Lavoie (1989) further addressed this issue by conducting an *in vitro* study. The authors reported that the growth of three micro-organisms commonly found in human wounds (Staphylococcus aureus, Escherichia coil, and Pseudomonas aeruginosa) was inhibited at the cathode resulting from exposure to HVPC for 2 hours at 250 V. Similarly, in their *in vitro* study, Szuminsky, Alber, Unger, & Eddy (1994) concluded that HVPC produced antimicrobial effects at 500 V when applied for 30 minutes. Although both studies demonstrated the efficacy of the bactericidal action of HVPC, the voltages used were too high to be tolerated by humans and may be detrimental to

collagen formation (Bourguignon & Bourguignon, 1991). Conversely, when Guffey and Asmussen (1989) compared the bactericidal activity of HVPC and LVDC in an *in vitro* system on Staphylococcus aureus, their findings demonstrated that HVPC did not result in bactericidal activity when current was applied for 30 minutes at voltages less than 160 volts. Typically, in a clinical setting, voltages of between 100 to 150 volts or less are employed (Alon, 1987; Nelson & Currier, 1991; Sussman & Bates-Jenson, 1998). Thus, if HVPC does exhibit bactericidal effects *in vivo*, it is unlikely these effects are the results of electrolysis.

The majority of research supports the use of cathodal current for the antibacterial effects, although there have been studies indicating bacterial inhibition at the anode (Bolton, Foleno, Means, & Petrucelli, 1980; Guffey & Asmussen, 1989; Loatsch, Ong, & Kloth, 1995; Ong, 1994). Loatsch, Ong & Kloth (1995) proposed two mechanisms by which cathodal DC stimulation directly decreases pathogens; first, the cathodal DC bombards organisms with electrons that continually excite cell membranes, thus depleting bacterial substrates and killing the organism, and second, that electrical stimulation disrupts intracellular metabolism. Ong, Laatsch & Kloth (1994) suggested that the documented antibacterial effects of continuous cathodal LVDC may be the result of galvanotaxic attraction (the attraction of cells to the anode or cathode) of phagocytic macrophages and leukocytes to the infected tissues rather than from the detrimental effects of pathogens caused by electrolysis or of alteration of the tissue pH. Guffey & Asmussen's (1989) study appears to lend support to this notion.

A recent literature review by this author reveals that to date, there remains a lack of published research articles on the subject of the antibacterial effects of HVPC conducted *in vivo*, indicating that there continues to be a need for projects of this nature.

CHAPTER III

MATERIALS AND METHODS

Subjects:

All studies to date that have been performed to determine the bactericidal effects of HVPC have been conducted *in vitro*. Previous authors agree that in order to truly establish the efficacy of the use of HVPC in the treatment of infected wounds, studies conducted *in vivo* are necessary (Guffey & Asmussen, 1989; Kincaid & Lavoie, 1989; Szuminsky, Alber, Unger, & Eddy, 1994). An animal model was chosen to eliminate any confounding variables associated with electrical stimulation treatment regimes used with human subjects (Fitagerald & Newsome, 1993). Previous animal studies conducted investigating the effects of electrical stimulation have used New Zealand rabbits (Girlanda, et al, 1982; Korpan, Resch, & Kokoschinegg, 1994; Riegels-Nielson, Espersen, Holmich & Frimodt-Moller, 1995; Rowley, McKenna, & Chase, 1974). Therefore, to maintain research consistency, New Zealand rabbits were used as subjects for this study. In order to satisfy the UMDNJ-RAF's Institutional Animal Care and Use Committee (IACOC) requirements, the methods were modeled after an existing research protocol. The study conducted by Rowley, McKenna, & Chase (1974) was chosen as the prototype for this project because it mirrored our project almost identically. The difference was that they were attempting to establish the antibacterial effects of LVDC *in vivo*.

This study involved the use of three series of six rabbits, for a total of eighteen. Three subjects were disregarded. Two because they were not sufficiently infected 24

hours post surgery (inclusion criteria required that the wounds be infected at a level greater then ten colony-forming units (CFU) per swab). The third rabbit was excluded because it partially removed its bandage causing the wound to dry out. The animals from the three series were analyzed as one group because the environmental conditions, number of treatment days, and data acquisition were all identical across series. Differences in environmental conditions, discrepancies in number of treatments due to scheduling difficulties, and the use of a different type bandage in an attempt to decrease the discomfort from the dressing changes resulted in the exclusion of animals from any additional series completed during this experiment. . The total number of animals used for this study was derived through a power analysis calculation (Appendix E).

The subjects included in this project were fifteen adult (4-6 months of age) New Zealand rabbits weighing 2 to 4 kg each. The animals were randomly assigned to an experimental group (EXP, n=7) and a control group (CON, n=8). The animals were placed in cages, one per specifically designed cage, and received 5 to 6 oz. of food daily and water ad lib. The temperature was kept constant at 22^0 and an equal ratio of daylight hours to non-daylight hours was maintained (12 hours on, 12 hours off).

Procedure

All fifteen rabbits were anesthetized, the hair clipped from their dorsum, and a 2 by 3 cm rectangular open wound, located one centimeter from the vertebral column midway between the scapular and pelvic areas, produced using aseptic surgical procedures. The wound was then infected by covering it with a sterile gauze pad soaked in 1 ml of $1X10^7$ Staphylococcus aureus solution (bacteria commonly found in wounds).

This solution concentration was chosen as previous studies have documented that when the bacterial content in an ulcer exceeds 10^5 organisms per gram of tissue, healing is impaired (Daltry, Rhodes, & Chattwood, 1981; Sapico, et al, 1986). The wound was then covered by a sterile dressing (Bioclusive™ film dressing) and the rabbit returned to the cage for post-operative recovery and observation.

Bacterial colony counts, measured in CFUs, were used to establish both the initial and final level of infection. Data was acquired from wound cultures obtained one-day post infection (initial) and six-days post treatment (final). The initial culture represents the wound immediately post infection and the final culture represents the wound after the treatment regime. Again, the subjects wound had to achieve a level of infection of greater then ten CFUs to included in this experiment.

UMDNJ-RAF's IACOC recommended against the use of tissue biopsy in order to prevent the animals from being subjected to any undue suffering. As a result, the quantitative swab technique was chosen as it is commonly used clinically and data show that tissue biopsy, needle aspiration, and qualitative swab techniques are comparable in terms of sensitivity, specificity, and accuracy (Stotts, 1995). The recommended method of performing the quantitative swab culture involves cleaning the wound with saline, placing the end of a sterile cotton-tipped applicator stick on a 1 cm^2 area of the open wound and rotating it. Pressure is applied to the swab to cause the tissue fluid to be absorbed in the cotton tip of the swab. The swab tip is then inserted into a sterile tube containing transport medium (Sussman & Bates-Jenson, 1998). The culture is then transported to the laboratory for analysis. Serial dilutions of the organisms are made on

agar plates. Results are expressed as organisms per swab or colony-forming units (CFU) per swab.

The experimental group received one hour of HVPC stimulation for six consecutive days from a Chattanooga Forte CPS 200 electrotherapy unit. On treatment days, the dressings were removed and electrodes constructed out of aluminum foil, wrapped in sterile gauze, and soaked in sterile saline were placed on all of the subject's wounds. Aluminum foil was chosen as the electrode material as it is an excellent conductor, non-toxic, inexpensive, disposable, comfortable, and can be sized as needed. The cathode was placed directly in the wound and the anode was placed 2 cm. caudal to the cathode. Both were secured by micropore paper tape and wrapped with an elastic bandage. Treatment parameters were as follows: the waveform was monophasic and twin peak in shape, the phase duration 75 usec, the pulse rate 100 pps, the amplitude the highest obtainable without causing muscular contraction (not to exceed 100 V), and the current modulation was continuous. These parameters are consistent with the parameters that are recommended clinically. The control group did not receive electrical stimulation; however, they did have similar electrodes placed in the wound for consistency on each of the sham treatment days. Both groups were restrained in a special apparatus (a cat-restraining bag) adapted for this procedure. After each treatment session, the wounds were redressed and the animals returned to their cages. In addition to the above procedures, the animals were checked daily, including weekends, for weight, food consumption, and temperature to ensure adequate nutrition and that the procedure did not result in sepsis of the animal.

Data Analysis

The Mann-Whitney U test, a test for ranked data when there are two independent samples, was used to compare the percent bacteria killed in experimental verses the control groups. The U test appeared to the most appropriate statistical analysis to use as it is suitable when the sample size is small and there are unequal data sets, as was the case in this project. Additionally, as is true for all tests for ranked data, the U test is immune to assumptions about normality and equal variances. Also, non-parametric statistical procedures are relatively unaffected by single outlier values. A standard alpha level ($p<0.05$) was selected. U tables supply critical values of U, which are determined by the number in each of the sample groups. Statistical significance is achieved when the observed U is less than or equal to the critical U.

A *t* test for two independent samples was conducted to compare the level of infection of the wounds in the EXP and CON groups in the PRE-treatment condition to ensure that there was no significant difference between the population means of the groups.

CHAPTER IV

RESULTS

Table 1 lists the results of the wound cultures, which are measured in colony forming units per swab (CFUs), for all subjects PRE-treatment and POST-treatment. The PRE-treatment condition represents the wound cultures for all subjects one-day post infection. The POST-treatment condition represents the wound culture after six consecutive HVPC treatment days for the EXP group and six consecutive sham treatment days for the CON group. The % change column represents the percentage of change of the wound colony count for the individual subjects from the PRE-treatment to the POST-treatment condition.

T-test analysis (Table 2) of the PRE-treatment experimental condition revealed that there was no statistically significant difference in the level of wound infection between the EXP and CON groups ($t = 1.65$, $p < 0.05$). A Mann-Whitney U test (Table 3) conducted compare the difference between the % change of the bacterial counts, revealed that there was no statistically significant difference between the EXP and CON groups from the PRE-treatment to POST-treatment experimental conditions ($U = 18$, $p < 0.05$).

Although statistical significance was not achieved, further assessment of the data presented in Table 1 revealed two interesting trends. First, when reviewing Table 1, we note that the EXP groups consistently exhibited a substantial decrease in their bacterial counts across all subjects and almost complete abolition in most subjects (mean decrease –97.4%, SE 1.36). Conversely, consistent results were not observed in the CON group; although some subjects did exhibit a marked decrease in their respective bacterial count,

others exhibited a substantial increase resulting in a mean increase of 22.5% (SE 75.56). This trend is made visually clear by viewing Fig. 3, which is a graphic representation of the % change of the wound bacterial counts from the PRE to POST-treatment conditions. Second, we note that the level of infection achieved in the wounds in the PRE-treatment condition varied across subjects in both the EXP and CON groups (Table 1). This response is consistent with past research that used an *in vivo* model (Rowley, McKenna, & Chase, 1974) and may be due to variations of the circulatory and immune systems of the individual animals. Due to this difference in the level of infection, a direct comparison of the changes that occurred may not be appropriate. However, when we convert this data into ranks and analyze the ranked data of groups PRE-treatment and POST-treatment (Table 3), we notice the data appears to exhibit an interesting general tendency. The mean rank of the CON group increased from 6.69 to 8.75, while that of the EXP group decreased from 9.50 to 7.14. This indicates that the subjects in the EXP group were infected at a higher level overall than the CON group in the PRE-treatment condition, yet in the POST-treatment condition the overall level of infection in the EXP group was less than that of the CON group.

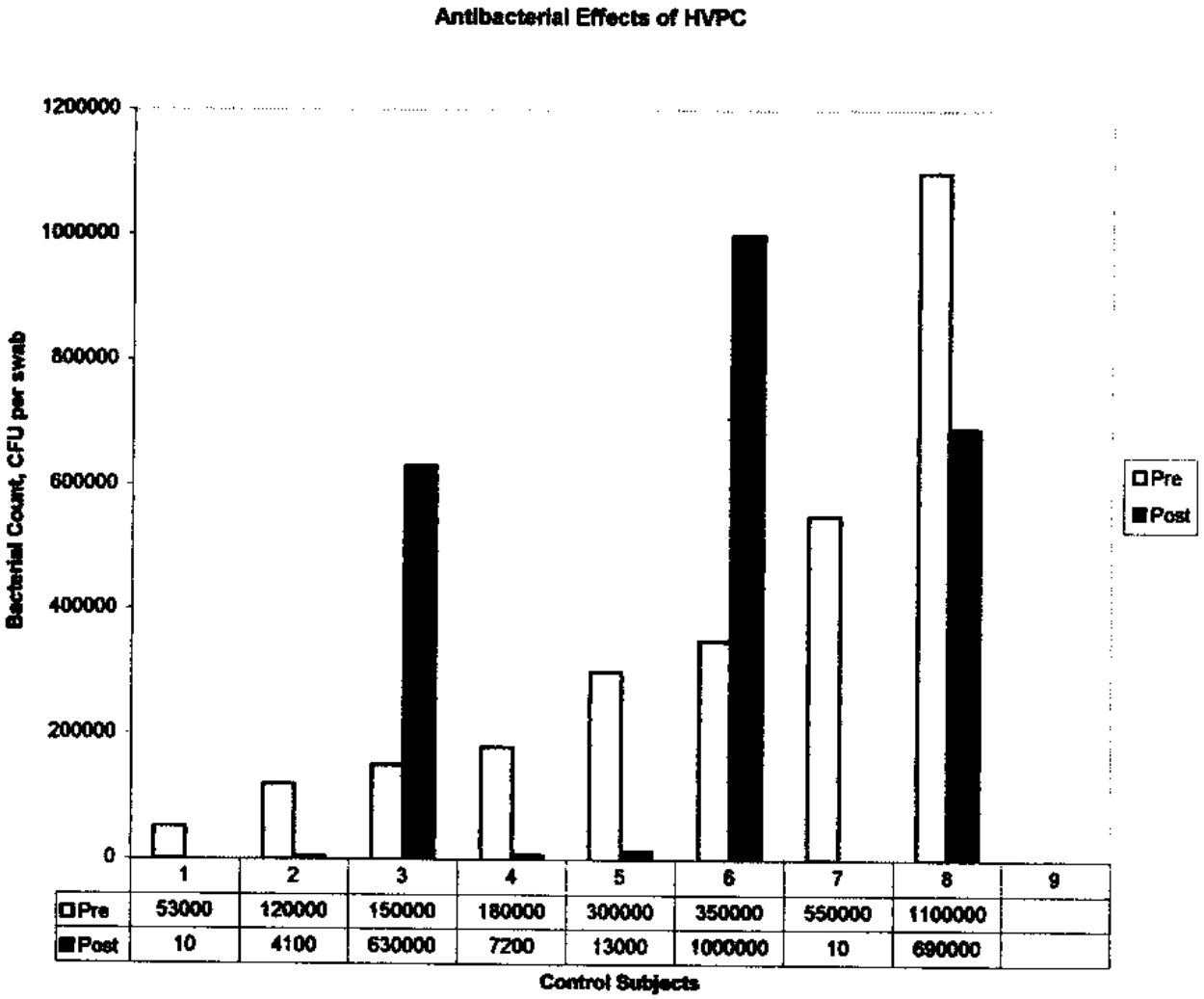
These two trends discussed may lend support to the notion that HVPC may have had a treatment effect, suggesting that HVPC stimulation may have decreased the bacterial effects greater in the EXP group when compared to the CON group, which received no electrical stimulation.

Table 1. Bacterial counts measured in colony-forming units ((CFU) per swab in the PRE-treatment condition (1 day post infection) and in the POST-treatment condition (after 6 daily treatments) and the % change from the PRE-treatment to POST-treatment condition.

Subject PRE-treatment POST-treatment

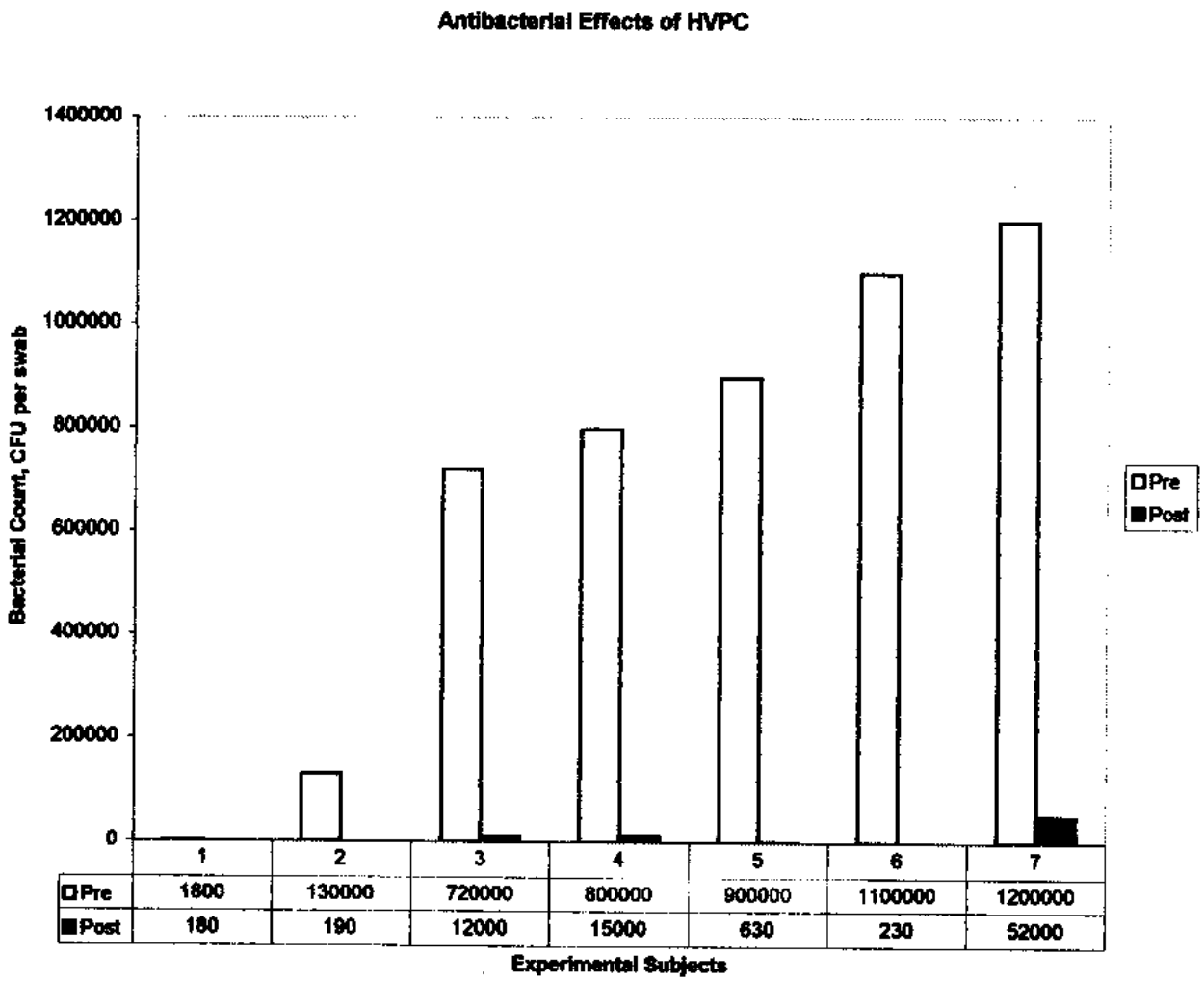
EXP GROUP	1 Day Post Infection	6 Day Post Treatment	% Change	Mean	SE
1 (00)	1,800	180	-90.0	-97.41	1.36
2 (01)	130,000	190	-99.85		
3 (46)	720,000	12,000	-98.33		
4 (50)	800,000	15,000	-98.12		
5 (02)	900,000	630	-99.93		
6 (11)	1,100,000	230	-99.98		
7 (45)	1,200,000	52,000	-95.66		
CON GROUP	**1 Day Post Infection**	**6 Day Post Treatment**	**% Change**	**Mean**	**SE**
1 (03)	53,000	10	-99.98	22.52	75.56
2 (47)	120,000	4,100	-96.66		
3 (48)	150,000	630,000	320		
4 (04)	180,000	7,200	-96.0		
5 (12)	300,000	13,000	-97.66		
6 (08)	350,000	1,000,000	271.43		
7 (05)	550,000	10	-99.99		
8(49)	1,100,00	690,000	-37.27		

Figure 1. Control Subjects: Bacterial count of the wound measured in colony forming units (CFU's) per swab in the PRE- treatment condition (1 day post infection) and the POST-treatment condition (after 6 sham treatment days).



	1	2	3	4	5	6	7	8	9
Pre	53000	120000	150000	180000	300000	350000	550000	1100000	
Post	10	4100	630000	7200	13000	1000000	10	690000	

Figure 2. Experimental Subjects: Bacterial count of the wound measured in colony forming units (CFU's) in the PRE-treatment condition (1 day post infection) and the POST-treatment condition (after 6 treatment days).



	1	2	3	4	5	6	7
Pre	1800	130000	720000	800000	900000	1100000	1200000
Post	180	190	12000	15000	630	230	52000

Figure 3. % Change comparison of the wound bacterial counts from the PRE-to the POST-treatment conditions of the experimental and control groups.

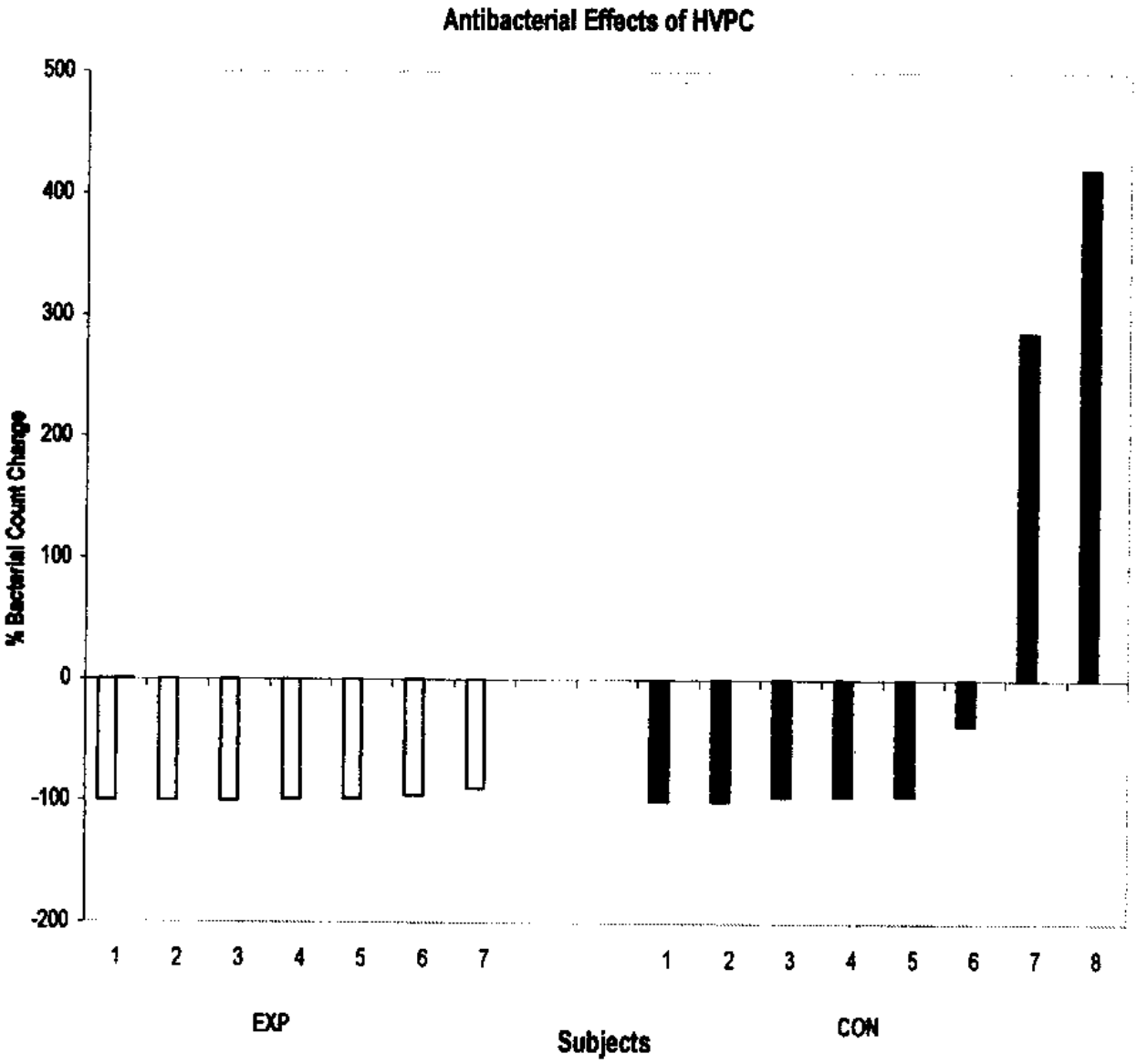


Table 2. Independent Samples T Test results.

		t	df	Sig. (2-tailed)	Mean Difference
PRE	Equal variances assumed	1.653	13	.122	342739.286
	Equal variances not assumed	1.618	10.978	.134	342739.286

Table 3. **Mann-Whitney U Test Results**

	Group	N	Mean Rank	Mann-Whitney U
Pre Test	Experimental Group	7	9.50	17.51
	Control Group	8	6.69	
	Total	15		
Post Test	Experimental Group	7	7.14	22.00
	Control Group	8	8.75	
	Total	15		
% Change	Experimental Group	7	6.57	18.00
	Control Group	8	9.25	
	Total	15		

CHAPTER V

DISCUSSION

The purpose of this study was to determine if HVPC, used at amplitudes human patients can tolerate, has an effect, *in vivo*, in reducing the viability of an infecting microorganism in wounds; thereby, positively affecting one of the extrinsic factors contributing to delayed wound healing. The results of this present project indicate that although significance was not achieved ($U=18$, $p<0.05$), there appeared to be a positive trend suggesting that HVPC stimulation decreased bacterial effect greater in the experimental group when compared to the control group, lending support for further investigation. Interestingly we note, while the experimental group exhibited a substantial and consistent decrease in their bacterial count across subjects, consistent results were not observed in the control group. While some of the controls did exhibit a substantial increase in their bacterial counts, others exhibited a marked decrease.

A possible explanation for the decrease in the bacterial counts in the control group may be that our subjects were young healthy rabbits who were receiving adequate nutrition and had intact circulatory, neurological, and immune systems thereby enabling some of the control subjects to combat the bacterial infection effectively on their own. The wounds produced on the subjects were acute, so one would expect they underwent a normal acute wound healing process. The biological repair process of acute wounds is based on four phases: inflammation, proliferation, epithelialization, and remodeling that occur in an orderly and overlapping fashion (Sussman & Bates, 1998). A detailed explanation of each of the four phases is beyond the scope of this discussion; however, the inflammation phase must be considered for this project. Inflammation is the body's

immune system reaction and is essential for healing. Acute inflammation begins at the moment of injury and the process lasts three to seven days; essentially the length of this project. During the inflammatory phase macrophages and neutrophils migrate to the wound site. Macrophages control infection by ingestion of microorganisms and excretion of ascorbic aced, hydrogen peroxide, and lactic acid. Neutrophils are granulocytic leukocytes that function as phagocytic cells that proliferate in the hypoxic acidotic environment and produce superoxide to fight bacteria. The neutrophil is considered to be a primary cell responsible for cleansing the wound of microorganisms, and lack of adequate numbers of neutrophils will retard healing of infected wounds. Thus it would appear that the control subjects who exhibited the substantial decreases in their bacterial count had strong enough immune systems to combat the infection during the inflammatory phase while the others who exhibited increases did not have sufficient means to deal with the increased bacterial burden. Conversely, the fact that all the subjects in the experimental group exhibited substantial decreases in their bacterial count would lead one to suspect that the HVPC treatment was a factor in promoting such consistent results.

The subjects in this study were young healthy animals; however, the human patients who will receive this treatment will be individuals who exhibit chronic wounds. These are patients whose wounds are ones that deviate from the expected sequence of repair time and response to appropriate treatment, as a result of a number of factors including skin changes that occur with aging, presence of chronic disease, circulatory disease, malnutrition, neuropathy, immune suppression and infection. These patients do

not have adequate intrinsic biological means to combat their infection and often require extrinsic assistance.

The use of HVPC clinically to promote healing of decubitus ulcers and surgical wounds initially based on the results of studies using LVDC; however, these are two separate waveforms and the results LVDC studies cannot be generalized HVPC. In response to this situation, Kincaid and Lavoie (1989) conducted an *in vitro* study and reported that the growth of three micro-organisms commonly found in human wounds (Staphylococcus aureus, Escherichia coil, and Pseudomonas aeruginosa) was inhibited at the cathode resulting from exposure to HVPC for 2 hours at 250 V. Similarly, Szuminsky, Alber, Unger, & Eddy (1994) in their *in vitro* study on similar organisms concluded that HVPC produced antimicrobial effects at 500 V when applied for 30 minutes. Although both studies demonstrated the efficacy of the bactericidal action of HVPC, the voltages used were too high to be tolerated by humans.

The purpose of our investigation was, therefore, to further study the question of the bactericidal properties associated with HVPC stimulation and to determine whether HVPC stimulation could inhibit or retard the growth rate of Staphylococcus aureus in an *in vivo* model at voltages that a human can tolerate. From the results of this present study, the author feels there may be a suggestion that HVPC stimulation of a wound infected with Staphylococcus aureus with parameters that are used clinically may result in decreased in bacterial growth *in vivo*. What we cannot state with certainty, however, is the mechanism of action by which HVPC appeared to exhibits these effects.

The work of Guffey and Asmussen (1989) demonstrated that HVPC applied at amplitudes ranging from 50 ma to 800 ma (corresponding to 10 to 160 V) did not result

in bactericidal activity *in vitro*. Therefore, we can assume that the voltage levels in our study were insufficient to kill the bacteria directly because the stimulation amplitudes did not exceed 100V. LVDC stimulators exhibit a polar capability, that is the ability to create a positive or negative electrical field; this is due to LVDC stimulator's monophasic waveform. It appears that it is the polar nature of the LVDC stimulators that give them their bactericidal and granulation tissue stimulating capability, as studies using symmetric biphasic waveforms which do not exhibit polarity did not yield similar results (Stefanovska, 1993; Baker, 1996). Past research has supported the effectiveness of HVPC in stimulating granulation tissue (Feeder & Kloth, 1985; Alon 1986, Kloth & Feeder, 1986; Unger, Eddy, & Raimastry, 1991). HVPC also has polar capabilities due to its monophasic waveform, and like LVDC, its granulation tissue healing capability appears to be related to its polar nature. Galvanotaxis is electrically guided cell locomotion due to polarity, or simply the attraction of cells to the anode or cathode. It would be reasonable to assume that if an HVPC stimulator is able to provide a sufficient enough electrical field to stimulate tissue growth due to its polar capabilities, it should be able to provide a strong enough electrical field to stimulate Galvanotaxis.

Electrically guided cell locomotion has been observed in a variety of cells including neutrophils and macrophages (Bourguignon & Bourguignon, 1989; Cooper & Schliwa, 1985; Fukushima & Sends, et al., 1953; Orida & Feldman, 1982). Perhaps the documented antibacterial effects of continuous cathodal LVDC and the suggested antibacterial effects of HVPC are the result of galvanotaxic attraction of phagocytic macrophages and leukocytes to infected tissues rather than from detrimental effects of

pathogens caused by electrolysis. Further studies analyzing the cellular motility effects of HVPC in infected wounds *in vivo* are necessary to address the mechanism of action.

CHAPTER VI

CONCLUSION

The purpose of this study was to determine if HVPC used at amplitudes that humans could tolerate had an effect in reducing the viability of an infecting microorganism in wounds. In this study we chose to use stimulating parameters that are consistent with the clinical treatment of chronic wounds in humans; that is, stimulation at the cathode for one hour at an amplitude of sensory stimulation not to exceed 100V. These were chosen in order to lend support to the use of these parameters clinically.

Although statistical significance may not have been achieved, the author felt that there was a suggestion that HVPC did appear to demonstrate a positive trend toward exhibiting bactericidal effects in acute wounds of animals. The lack of statistical significance may have been do to the use of a healthy animal model with an acute wound; however, use of sick animal model with a chronic would have been infeasible, if not inhumane. In view of this, the author feels that further studies using animals may not be appropriate due to the lack of generalizability, and suggests it would be more appropriate to conduct further studies on human subjects.

Further studies comparing the outcome of homogeneous groups patients with infected chronic wounds who received standard treatment with the outcome of those who receive standard treatment plus HVPC would be necessary to truly establish the efficacy of the antibacterial effects in humans and lend support to its clinical use. Additionally, further studies involving the analysis of human cell locomotion in response to HVPC are required in order to determine the mechanism of action of the bactericidal effects.

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APPENDIX A

ABSTRACT: The following abstract was accepted by both the NJAPTA and the 1999 APTA National Annual Conferences for poster presentations.

ANTI-BACTERIAL EFFECTS OF HVPC *IN VIVO* Campolo M, Pinto-Zipp G, Ryden E, Addario E, Hughes K Graduate Program in Health Sciences and Research Animal Facility UMDNJ-Seton Hall, Newark, NJ, USA

PURPOSE: A historical review of the literature reveals that low intensity direct current (LIDC) is effective in the treatment of infected wounds. Since the 1970's, high voltage pulsed current (HVPC) stimulators have been used for the same purpose based on the assumption that they have the same physiological effects as LIDC. To date; however, there is insufficient research to support clinical use of HVPC for infected wounds. The purpose of this pilot study was to determine whether HVPC has an inhibitory effect on bacteria *in vivo* in order to provide evidence to support the clinical use of HVPC stimulation in the treatment of infected wounds.

SUBJECTS: An animal model was used in order to avoid any of the confounding effects associated with the use of human subjects. All the rules and regulations set forth by the Institutional Animal Care and Use Committee were strictly adhered to. IRB approval was also obtained. The subjects consisted of twelve New Zealand rabbits of equivalent size, weight and age.

METHODS AND MATERIALS: The animals were randomly assigned to either an experimental group (EXP=6) or a control group (CON=6). Each animal was anesthetized and a full thickness wound (2cm by 3cm) was made on their backs, which then was infected with 1 ml of 1 x 10^7 Staphylococcus Aureus solution. The wound was covered with a sterile dressing and sampled at 24 and 48 hours to ensure it was sufficiently infected. Electrodes were placed on all of the animals; however, only the EXP group received electrical stimulation. The parameters chosen were consistent with those recommended clinically: the waveform was monophasic and twin peak in shape, the phase duration was 75 usec, the pulse rate was 100 pps, the amplitude was the highest obtainable without causing muscular contraction (not to exceed 100V), and the current modulations was continuous.

RESULTS: The data were analyzed using the Mann-Whitney U Test, due to the small sample size, which revealed significance at the 0.05 level. The EXP group exhibited a consistent and substantial decrease in their bacterial count across all subjects, with a resulting mean decrease of 98%. Conversely, consistent results were not observed in the CON group. While some subjects of the CON group did exhibit a decrease in their bacterial count, others exhibited a substantial increase, resulting in a mean increase of 112%. These results suggest that HVPC stimulation decreased bacterial effects greater in the EXP group when compared to the CON group, which received no electrical stimulation.

CONCLUSION: HVPC appeared to exhibit bactericidal effects when used with parameters that are consistent with the clinical treatment of chronic wounds. Further studies using larger sample sizes are necessary to support the results of this study.

APPLICATION TO PHYSICAL THERAPY: This pilot study appears to lend support to the clinical use of HVPC by physical therapists in their treatment of infected chronic wounds.

APPENDIX B

Letter of Acceptance from the Institutional Animal Care and Use Facility

UMD
NEW JERSEY

NEW JERSEY MEDICAL SCHOOL

The Institutional Animal Care
and Use Committee (IACUC)
Phone: (201) 982-4669
Fax: (201) 982-2620

185 South Orange Avenue
MSB A-602
University Heights
Newark, NJ 07103-2714

MEMORANDUM

DATE: December 10, 1998

TO: Marc Campolo, M.A., P.T., S.C.S.
Associate Professor
Physical Therapy

FROM: Lois B. Laemle, Ph.D., Chair
Eva B. Ryden, Ph.D., D.V.M., Attending Veterinarian
Institutional Animal Care and Use Committee

RE: **Annual Renewal:**
Animal Care and Use Protocol # 0759
Title: Anti-Bacterial Effect of HVPC *in VIVO*

Your animal care and use protocol for the project shown above was reviewed and approved by the Institutional Animal Care Use Committee on December 8, 1998.

This approval will remain in effect until: 01/27/00.
Original approval date for this protocol: 01/27/98.
Protocol may be continued by annual updates until: 01/27/01.

Federal laws and guidelines require that Institutional Animal Care and Use Committee reviews ongoing projects annually. For the first two years after initial approval of the protocol you will be asked to submit an annual update form, describing any changes in procedures or personnel. The committee may, at its discretion, extend approval on the project in one year increments until the third anniversary of the original approval of the project.

Approval may only be extended until the third anniversary of the original approval of the project. At that time, the protocol must be replaced by an entirely new submission.

EBR/dp

APPENDIX C

Application to use lab animals at UMDNJ Newark, submitted October 1997.

APPLICATION TO USE LABORATORY ANIMALS AT UMDNJ-NEWARK

(FOR IACUC USE ONLY)

Date Submitted: October 10, 1997

Project #

Category: A B (C) D
(please check appropriate choice)

Classification:
New
Modified
Renewal

1) PRINCIPAL INVESTIGATOR: Marc Campolo, MA,PT,SCS

Academic Title: CLINICAL ASSOCIATE PROFESSOR

Department: PHYSICAL THERAPY

Address: 65 BERGEN STREET, MARTLAND BUILDING, NEWARK Phone: 973-972-5272

2) PROJECT TITLE: ANTI-BACTERIAL EFFECT OF HVPC IN VIVO

Source of funding: UMDNJ Physical Therapy Department

Duration of grant - starting date: **ending date:**

Grant identification #(or 'pending')

3) **PLEASE PROVIDE NAME, TITLE, PHONE# (OFFICE AND HOME) OF ALL INDIVIDUALS WORKING WITH THE ANIMALS.**

a.	Marc Campolo	Clinical Associate Professor	717-476-5564
b.	Kathleen Hughes	Physical Therapy Student	908-232-5669
c.	Tom Kenny	Physical Therapy Student	973-759-4552
d.	Ellen Splaine	Physical Therapy Student	732-671-4969
e.	Kim Yarashefski	Physical Therapy Student	973-635-1899

4) **RESEARCH PROTOCOL**

Please discuss the research protocol. It should include: specific aims, background, the experimental design/methods and the potential benefits to humans and/or animals.

Specific aims:

The purpose of this study will be to determine if high volt pulsed current (HVPC) has an effect, in vivo, in reducing the viability of an infecting microorganism in wounds at levels human subjects can tolerate.

Background:

Early research studies have reported the efficacy of low intensity direct current (LIDC) in facilitating wound healing in both animals and humans. The reasons that were cited are the bactericidal effects of electrical current and the stimulation of granulation tissue growth. Since the 1970's, clinicians have been treating chronic wounds with high voltage pulsed current based on the mistaken assumption that they are similar to the LIDC stimulators and thereby exhibited the physiological effects commonly attributed to LIDC stimulation. The assumed antibacterial and granulation tissue effects of HVPC were based almost entirely on the results obtained in LIDC studies.

Experimental design/methods:

Thirty rabbits will be randomly assigned to either an experimental group (n=15) or a control group (n=15). The animals will receive a full-thickness wound which will be infected with a bacterium commonly found in chronic dermal wounds. The experimental group will be exposed to HVPC in order to determine the effect it has on the bacteria. The subjects will receive the treatment for seven consecutive days and the wounds will be sampled for ten consecutive days to establish both the efficacy of the treatment and whether there is any latent effect. The data will be analyzed using appropriate statistical analysis.

Benefits:

Previous research suggests that HVPC is as effective as LIDC in augmenting wound healing. Research has suggested that electrical stimulation enhanced rate and extent of healing of chronic wounds. Based on information currently available, high volt treatment has been shown to be more cost effective and less time consuming.[1,5,13,22,25,33,34,36,49]

5) ANIMALS TO BE USED IN THIS STUDY.

	Species Strain	Sex	Weight/Age	Annual Quantity	Total
a.	New Zealand Rabbits	Female	1.3-1.8 kg/4-6 months	30	30

b./c.

d. Will breeding be done? Yes (No) If yes, indicate which species.

e. Based on your experimental design, indicate how the total number of animals to be used was derived.

Both the control and the experimental groups will have fifteen animal subjects. Based on previous experiments using rabbits as the animal subjects, fifteen was determined to be a sufficient number for a pilot study.[35,47,49]

6) JUSTIFICATION FOR THE USE OF ANIMALS. Note: if this section is not complete, the application will not be processed.

a. Indicate the rationale for using animals.

Animals were chosen to eliminate any confounding variables associated with stimulation treatment regimes used with human subjects.[34]

Previous research of the anti-bacterial effects of electrical stimulation have been animal studies using New Zealand rabbits. In order to maintain research consistency, New Zealand rabbits were chosen as subjects for this study.[27,35,47,49]

b. Why was this species chosen?

The New Zealand white rabbits were chosen in order to maintain research consistency.[35,47,49]

c. Provide specific information verifying that the research is not unnecessarily redundant or repetitious.

All the studies to date that have been performed to determine the bactericidal effects of HVPC have been conducted in vitro. All the authors agree that in order to truly establish the efficacy of the use of HVPC in the treatment of infected wounds, studies conducted in vivo are necessary.[28,31,38]

d. For all procedures that involve momentary or slight pain (survival and nonsurvival surgeries are procedures in which alternatives must be considered), provide specific information that demonstrates that alternatives to the use of animals have been considered and are not possible. Information regarding which procedures may cause momentary or slight pain, please refer to guidelines page B.

Ample studies have been conducted in vitro.[16,28,31,36] In order to progress the research, studies must now be performed in vivo on animal subjects.

As previously stated, the use of humans would present too many confounding variables. Due to these facts, animal subjects appear to be appropriate for this study.

e. What sources or databases were searched, please check the appropriate item(s) including but not limited to:

Medline ✓ Animal Welfare Information Center ✓ Other(specify title) OVID ✓

f. Provide the key words used in the search listed above.

rehabilitation, Transcutaneous Electrical Nerve Stimulation, staphlococcus, HVPC, humane, LIDC, anesthesia, Galvanic, wound, electrical stimulation therapy, electrical stimulation, rabbits

7) **EXPERIENCE AND TRAINING**

a. **Describe your training and experience with the procedures and techniques to be used on the animals you will be using in this protocol. If you are inexperienced in these procedures, describe how you will obtain the appropriate training. Please attach one copy of your Curriculum Vitae to the original application.**

Marc Campolo, MA, PT, SCS
-Clinical Associate Professor at UMDNJ, SHRP MPT Program
-Electrotherapy Professor
(please refer to attached Curriculum Vitae)

b. **List all individuals (include UMDNJ position titles) who will be involved in the use of animals and indicate their experience with the experimental procedures. If those individuals are inexperienced indicate your plans for directly supervising them during training.**

Marc Campolo	Clinical Associate Professor
Kathleen Hughes	Physical Therapy Student
Tom Kenny	Physical Therapy Student
Ellen Splaine	Physical Therapy Student
Kim Yarashefski	Physical Therapy Student

Students will be directly supervised by Clinical Professor.
All individuals listed above will attend the RAF orientation seminar.

8) **ANIMAL PROCEDURES**

a **AREAS IN WHICH PROCEDURES WILL BE PERFORMED**

Research Animal Facility (Yes) No
If not RAF, state location

b. **TYPE OF PROCEDURE**

Acute(<5 days)
Chronic **Length** 10 days
Deprivation **Length**
Restraint **Length**
Animal used as tissue source only Yes (No)

c. **PHYSICAL DISCOMFORT**

None
During procedure only
Immediately following procedure
Long term possibly long term, due to wound infection.
Other

Clinical condition or abnormality expected: We would expect to see a decrease of Staphlococcus Aureus in the wound.

Measures that will be used to alleviate discomfort:
Specify drug buprenophine **Dose** 0.02mg/kg **Route** intramuscular
Administered by: appropriate personnel
The rabbits will be premedicated with buprenophine to confer preemptive analgesia. Rabbits will be observed daily including weekends. If animals lose more than 20% body weight, become anorexic, or if body temperature exceeds 105 for more that 24 hours, the rabbit will be euthanized.

d. Outline clearly, specifically and concisely the experimental usage, aseptic techniques and procedures to be used on the animals. If surgery is involved then also answer Question 9. (Attach one page if necessary) The following protocol has been utilized in previous studies. It appears appropriate for this project.[16,25,31,35,36,38,47,49]

The animals will be randomly assigned a number and will be placed in cages, one per cage. The rabbits will receive both food and water ad lib. Temperature(22^{0}C) and the ratio of daylight hours to non-daylight hours (12 hours dark/12 hours light) will be kept constant. The animals will be divided into two equal groups, experimental (n=15) and control (n=15). The experimental group (Exp 1) will have the wound infected with a microorganism commonly found in chronic wounds (such as staphylococcus aureus) and will receive HVPC stimulation by having an electrode placed directly in the wound. The control group (Con 1) will have the wound infected and will have an electrode placed in the wound, however will not receive HVPC stimulation. The electrode will be placed in the control groups wound to determine whether the electrode itself would have an antibacterial effect. The apparatus will be a HVPC stimulator. Commercial supplier, model number, and suppliers name and location will be determined prior to initiation of the research project.

The rabbit will be anesthetized. The hair will be clipped from the dorsum of the animal. The area will be aseptically prepared. Aseptic surgical procedures will be used in producing the required wound. A one centimeter by three centimeter rectangular incision will be made longitudinally one centimeter from the vertebral column, midway between the scapular and pelvic areas. The skin and underlying muscle fascia will then be removed producing a one centimeter by three centimeter open wound.

The wounds will be infected by placing a sterile gauze pad that has been soaked in one milliliter of dilute culture of staphylococcus aureus on the wound. The wound will then be covered by a sterile gauze pack, which will be soaked with sterile distilled water. The whole area will then be bandaged. The rabbit will be returned to the cage for post-operative recovery and observation. After twenty-four hours, each wound will be sampled to ensure that it has been infected.

Carbon filled silicone electrodes will be chosen to reduce any confounding antibacterial effects from the use of metallic electrodes. The electrodes will be wrapped in sterile gauze and soaked in sterile saline. The cathode will be placed directly in the wound and the anode will be placed at least 2 cm proximally to the cathode on the rabbits shaved skin. The electrodes will be secured by micropore paper tape and then wrapped with an elastic bandage. The animals in the control group will receive on hour of HVPC daily for seven consecutive days. The treatment parameters are as follows: wave form monophasic (typically the characteristic twin peak monophasic wave form), phase duration is predetermined by the manufacturer and is usually in the 50 to 100 usec range, pulse rate will be set at 100 pps, polarity is negative for the treatment electrode, amplitude will be set at sensory stimulation sub-threshold to muscle contraction, and current modulation mode will be continuous. These parameters were chosen because they are consistent with the parameters that are recommended clinically. The subjects will receive this treatment for seven consecutive days.

Prior to each treatment, the dressing will be removed and the wounds will be sampled. The wounds will also be sampled on days eight through ten to determine if there are any latent effects. The wounds will be sampled by use of a sterile cotton swab dipped in a tube containing 5 ml of sterile trypticase soy broth. The swab will be rolled for one revolution across the surface of the wound and replaced in the tube. The sample will be sent to the medical laboratory for analysis to determine the relative number of organisms present on each wound as well as white blood cell count. In addition, the animals will be checked daily including weekends for weight, temperature and food consumption.

9) SURGICAL PROCEDURES NOTE: Before completing this section, you must consult with and RAF veterinarian. No trade names will be accepted for drug names.

a. PRE-OPERATIVE PERIOD

Fasting required (No) Yes Length

b. ANESTHESIA

Pre-anesthesia N/A
Drug Dose Route
Drug Dose Route
Administered by:

Induction:
Drug Ketamine Dose 35 mg/kg Route intramuscular
Drug Xylazine Dose 5 mg/kgRoute intramuscular
Drug Buprenet Dose 0.02 mg/kg Route intramuscular
Administered by:

Anesthesia: Same as above
Drug Dose Route
Drug Dose Route
Administered by:

c. INTRA-OPERATIVE PERIOD

Survival Non-survival
Location

Describe the surgical procedure. Include provisions for asepsis, and who will perform the surgery. See above

d. POST-OPERATIVE PERIOD

e. Describe postoperative care including analgesia, other medications and the name of person responsible. If you are using analgesia, please justify.

Drug Dose Route
Drug Dose Route
Administered by: Preemptic. Post operative analgesic not expected to be required.

10) REQUIREMENTS FOR THE PROJECT

a. AREA ANIMALS TO BE HOUSED: at RAF UMDNJ-Newark
Routine Housing

b. HEALTH PROBLEMS

In case of animal health problems, it may be necessary to administer drugs such as antibiotics or steroids to your animals. Can any drug be used to treat experimental animals? Yes (No)
Do not use the following drugs due to interference with study results:
Antibiotics

c. **WEEKEND/HOLIDAY CARE**

If it is necessary for the post-operative or post-procedural care to extend over a weekend or holiday, who will be the responsible individual(s).

Name	Phone
Marc Campolo	717-476-5564
Kathleen Hughes	908-232-5669
Tom Kenny	732-759-4552
Ellen Splaine	732-671-4969
Kim Yarashefski	973-635-1899

d. **IN CASE OF SUDDEN DEATH OF ANIMAL(S) THE COURSE OF ACTION TO BE FOLLOWED.**

Dispose of Carcass	Yes	(No)
Hold Carcass in cold storage	(Yes)	No
Notify Investigator	(Yes)	No
Necropsy	Yes	(No)

11) **EUTHANASIA**

Describe the final disposition of all of your animals. Describe the exact method, if using anesthetics list agent, dose, and route of administration. (Note: Method of euthanasia shall follow current guidelines established by the American Veterinary Medical Association Panel on Euthanasia AVMA Vol. 202, No. 2, 1993). If these guidelines are not to be followed provide a scientific justification. If using cervical dislocation or decapitation, provide a scientific justification.

Method
Agent Beothanasia **Dose** 1 ml/10lbs **Route** intravenous

12)**BIOHAZARDOUS AGENTS**

a. **Are you using a volatile anesthetic (e.g. ether or halothane)?** Yes (No)

If yes, state which anesthetic, where and how will this be vented?

b. **Can the natural/experimental disease or pathological condition under study be transmitted to humans?** (Yes) No

If yes, what precautions are you taking to protect persons who will come in contact with these animals? Universal precautions and sterile techniques

c. **Check the appropriate items. Are carcinogens , infectious agents , toxins , or other biohazardous materials used in live animals?** (Yes) No

If yes, Specify agent, dose, route of administration and frequency. Please attach a copy of the Biosafety Approval letter.

d. **Are radioisotopes used in live animals?** Yes (No)

If yes, specify agent, dose, route of administration and frequency. Please attach a copy of the Radiation Safety Approval letter.

13) ASSURANCES

I pledge to conduct this project in accordance with the intentions set forth in this application. If I wish to make any substantive alterations during the course of the project, I will submit a written request or approval of new procedures. I assure that the animals requested for this project will be used in accordance with the provision of the Animal Welfare Act and the guidelines and policies approved by the IACUC, as described in the UMDNJ-Newark RAF GUIDE.

DATE 10/21/97

SIGNATURE OF PRINCIPLE INVESTIGATOR

SIGNATURE OF DEPARTMENT CHAIRPERSON

Alma Merians 1/6/98 BR

SIGNATURE OF GRADUATE'S MENTOR SIGNATURE

DATE 1/25/98

APPENDIX D

Protocol for the use of infectious agents in animals.

UMDNJ-NEW JERSEY MEDICAL SCHOOL

Protocol for the Use of Infectious Agents in Animals

This document is to be completed whenever an animal research protocol involves the use of etiologic agents. Contact Environmental and Occupational Health and Safety Services (EOHSS) at 2-4812 if you desire assistance in completing this application.

Date Submitted: October 10, 1997

Principal Investigator: Marc Campolo, MA, PT, SCS

Title of Protocol: Anti-Bacterial Effect of HVPC In Vivo

BIOHAZARDOUS AGENT

Organism Staphlococcus Aureus Animal Pathogen? Yes

CDC/NIH Biosafety Level* BSL-1 Animal Biosafety Level BSL-1

Natural Route(s) of Infection (vector?) Transcutaneous

Experimental Administration Route, Volume, and Titer On skin 1 x 10^4 bacteria/ml

Is Agent Transmitted from Animal to Animal? Yes

Is Agent Transmitted for animals to humans? Yes

Is There an Available Vaccine and/or Therapy? Yes - Antibiotics

Will Organism Be Inactivated Prior to Use in Animals? No

Disinfectant of Choice Clidox

Is the agent a particular hazard for immunodeficient individuals or for others whose underlying medical status may put them at increased risk, e.g., certain microbes (Listeria, Toxoplasmosis) pose a particular risk to fetal health?

Yes

*Biosafety Levels refer to the risk designations found in either the CDC/NIH's *Biosafety in Microbiological and Biomedical Laboratories* or the recent version of the NIH's *Guidelines for Recombinant DNA Research*. These publications are on file at EOHSS.

HOUSING OF ANIMALS AND EXPERIMENTAL PROTOCOLS

A. RODENTS (small animals)

Will rodents be housed in microisolator cages?

Will procedures be performed in a Biological Safety Cabinets? (Cabinets must be certified annually.)

B. NON-RODENTS (larger animals)

What precautions, if any, need to be taken to contain infectious materials given the inability to use the types of containment devices available for use with rodents and other small animals?

Cages/papers will be disposed in double black bags. .Personnel will wear masks, gowns and gloves.

C. BARRIER HOUSING Not Needed

"Barrier Housing" refers to the use of engineering controls [air filtration, air pressure differentials], and work practices to "protect the animal from the environment". Such a system would for instance be applicable when working with SCID mice and other situations where exposure to "typical" environmental stressors might compromise experimental integrity.

Will infected animals be housed under barrier conditions?

Will procedures be performed under barrier conditions?

D. EXPERIMENTAL PROTOCOL

Describe the animal-experiment procedures that will involve the use of this agent.

Animals will receive a topical application of Staphlococcus Aureus on a surgical wound. See IACUC protocol attached.

To what extent will viable organisms be shed into the environment by way of excreta, open skin lesions, exhalation, saliva, or nasal secretions?

Note the type of Personal Protective Equipment to be used.

Have all project personnel demonstrated to the Principal Investigator's satisfaction adequate knowledge of the hazards associated with the particular etiologic agent(s) and animal handling techniques appropriate to minimize the risk of infection?

EMERGENCY PROCEDURES

Describe what will be done:

1. In the event of overt personal exposure:
   Area of skin exposed would be washed thoroughly. Personnel will be directed to seek appropriate medical attention.

2. In the event of overt environmental contamination:
   All surfaces will be thoroughly cleaned with a 10% bleach solution.

DECONTAMINATION AND DISPOSAL PROCEDURES

1) Decontamination (animal cages, room surfaces, instruments):

   Regular cage wash.

2) Disposal procedures:

   carcasses Double bagged in black bags.

   bedding, other disposable materials
   Double bagged in black bags.

I acknowledge and accept responsibility for the conduct of this research at Biosafety Level _____ as approved by the UMDNJ Newark Biohazard Subcommittee. I shall also be responsible for notifying the Committee of any changes in the protocol involving the handling, storage, or disposal of infectious agents.

Principle Investigator

Department Chair's Signature/Date 1/25/98

Chair, Biohazard Subcommittee

Graduate Student Mentor's Signature/Date 1/25/98

APPENDIX E

Power analysis Study

>From harten Thu May 27 09:54:04 1999
X-Sender: harten@njmsa.umdnj.edu
Date: Thu, 27 May 1999 09:58:01 -0400
To: campolma@umdnj.edu
From: Bob Harten <harten@UMDNJ.EDU>
Subject: Power analysis

Marc:

I finally got run some tests on your data. It looks pretty good! As it stands with an N of 5, depending on the Alpha value you choose (.01 or .05, both of which are amply high, I would go with .05) your present study has a power of 0.75 and 0.90 respectively. These are very good numbers. I ran the test to determine N #'s for varying powers and alphas which is attached. Based on the present study, groups of 8 are excellent. The statistics with this number are very strong and, in my opinion, beyond reproach. I hope that this is of some help, and again I appologize about the delay in getting this together. Feel free to get in touch with me if you have any questions.

Good luck on your project

Bob Harten
Attachment Converted: C:\INTERNET\EUDORA\ATTACH\Mc1.rtf

Attachment Converted: C:\INTERNET\EUDORA\ATTACH\Mc2.rtf